Paradoxical Effect of Troglitazone in Normal Animals
Enhancement of Adipocyte but Reduction of Liver Insulin Sensitivity

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Troglitazone is an antidiabetic agent that improves the ability of adipocytes to store triglycerides by enhancing their insulin sensitivity. Although potent in insulin-resistant states, the effect of troglitazone on lipid and glucose turnover in normal animals has not been assessed. Euglycemic clamps were performed as an insulin dose response in normal mongrel dogs (n = 6). Somatostatin was infused without hormone replacement (zero insulin) for 90 min. Insulin was then either portally replaced (1.8 pmol · min–1 · kg–1), overreplaced (5.4 pmol · min–1 · kg–1), or overreplaced peripherally to match the systemic levels of the portal overreplacement dose (2.3 pmol · min–1 · kg–1) for 180 min. A total of 600 mg troglitazone was then given orally each day for 3 weeks and continued throughout a second experimental phase, at which point the euglycemic clamps were repeated. In concordance with previous studies, endogenous glucose production (EGP) was similar whether insulin was delivered portally or peripherally, both before and during troglitazone treatment. Although free fatty acids (FFAs) at zero insulin were not affected, there was a leftward shift of the insulin-FFA dose response curve secondary to a suppression of FFA release into plasma. EGP was paradoxically elevated by troglitazone treatment because of an elevation of both gluconeogenesis and glycogenolysis. In conclusion, troglitazone reduced hepatic sensitivity to FFAs. Because EGP is a primary determinant of fasting blood glucose, we hypothesize that a protective mechanism exists in normal animals, preventing hypoglycemia during insulin sensitization with troglitazone. Diabetes 49:2087–2093, 2000

More than 15 million people in the U.S. are afflicted with type 2 diabetes. Of these, >80% are also obese. Reduction of obesity by diet and exercise can be instrumental in combating obesity-linked diabetes. However, the link between obesity and diabetes is not fully understood. Examination of the effect of pharmacological agents affecting adipose tissue may help clarify the role of adiposity in the pathogenesis of type 2 diabetes.

One group of agents that appears to ameliorate type 2 diabetes by acting on adipocytes are the thiazolidinediones. Troglitazone is an oral antidiabetic drug of the thiazolidinedione class. Although the specific mechanism of action of thiazolidinediones to improve glucose tolerance is not known, it is likely that they act by stimulating the peroxisome proliferator–activated receptor (PPAR) class of nuclear receptors (1). Of the known PPAR isoforms, troglitazone binds with the highest affinity to PPAR-γ, which has been shown by mRNA analysis to be distributed primarily in white fat tissue (2).

PPARs have been implicated in controlling fat metabolism (3). Pharmacological ligands to PPAR-γ have been shown to promote adipocyte development in vitro (4), as evidenced by increased fat storage. In vivo, activating PPAR-γ improves sensitivity of muscle tissue to insulin (5). A proposed mechanism by which thiazolidinediones, with their receptors in white fat tissue, enhance insulin sensitivity in muscle tissue emerges from the Randle hypothesis (6), which proposes an inverse relationship between fatty acid and glucose oxidation. Thiazolidinediones may enhance sensitivity of the adipocyte to insulin, lowering lipolysis and thereby enhancing the storage of lipids. The consequential reduction in plasma free fatty acids (FFAs) could lead to enhanced glucose uptake and/or utilization by muscle tissue.

In contrast to the effect on muscle tissue, the effect of troglitazone on glucose production by the liver is poorly understood. At the highest clinical dose of troglitazone in type 2 diabetic humans (600 mg/day), fasting FFAs and endogenous glucose production (EGP) are suppressed (7). Our laboratory has proposed that insulin’s primary effect to control glucose production is through an indirect pathway involving FFAs; insulin suppresses lipolysis at the adipocyte,
which lowers plasma FFA. We and others have presented evidence that it is the lowering of FFAs that signals the liver to suppress glucose production (single gateway hypothesis) (8–10). In light of this concept, it is possible that enhancing adipocytic insulin sensitivity improves the ability of insulin to suppress lipolysis, which could suppress EGP through reduced plasma FFAs. This latter possibility was tested in this study in normal animals of normal body adiposity under conditions of hypoglycogonemia, in which this indirect pathway has been demonstrated to be enhanced (11). The effect of chronic sensitization of the adipocyte to insulin on EGP and insulin-stimulated glucose uptake was thus assessed.

RESEARCH DESIGN AND METHODS

Animals. Experiments were performed on six male mongrel dogs (26.7 ± 2.2 kg). The maintenance and surgery of the animals have been previously described (8). Chronic catheters were implanted at least 7 days before the first experiment. One catheter was inserted into a carotid artery for sampling of arterial blood. A second catheter was placed in the femoral vein and advanced to the inferior vena cava for infusion of tracer, somatostatin, and insulin. A third catheter was placed in the portal vein 4 cm upstream from the porta hepatis for portal infusion of insulin. On the day of the experiment, an acutely placed catheter was placed in the saphenous vein for glucose infusion, and another catheter was placed in the saphenous vein of the contralateral leg for peripheral insulin infusion. Plasma liver enzymes were measured every 4 weeks as recommended for troglitazone treatment in humans (12).

Experimental protocol. Three euglycemic clamp protocols were performed in each dog before troglitazone therapy, and three identical clamps were performed after troglitazone therapy. Protocols were separated by at least 5 days and performed in a randomized order.

Before troglitazone. The two portal infusion protocols done on every dog included a zero dose of insulin as well as one of two other doses (low portal [LPO] or high portal [HPO]) administered into the portal vein (1.8 or 5.4 µmol · min⁻¹ · kg⁻¹). In four of the six dogs, we also performed a clamp study in which we gave an intermediate dose of insulin administered peripherally (intermediate peripheral [IPE], 2.3 µmol · min⁻¹ · kg⁻¹) into a saphenous vein. IPE administration into a peripheral vein was designed to match peripheral insulin levels obtained with the HPO dose; however, the portal insulin is expected to be lower with IPE administration. Finally, in four of the six dogs, an additional study was performed in the basal condition in which tracer was infused but without hormonal intervention. Thus, before troglitazone administration, 20 experiments were performed (6 dogs × 2 protocols + 4 dogs × 2 protocols).

With troglitazone. After the initial series of protocols, 600 mg troglitazone was given orally once per day with the daily meal over a period of 6 weeks. Thus, the 20 experiments described above were performed over 15 days, after which troglitazone was administered for the following 3 weeks. Drug treatment was continued for 3 more weeks, during which the abovementioned 20 experiments were repeated.

Euglycemic clamp protocol design. All clamps were done as follows. At ~180 min, a bolus of either deuterated water as normal saline (10.5 ml/kg body wt, LPO protocol only; see below); Sigma-Aldrich, St. Louis, MO) or normal saline was infused into the femoral vein over a period of ~30 min. At ~120 min, tracer glucose was infused (25 µCi/µmol, 0.25 µCi/min/µmol [3-3H]-glucose; NEN Research Products Du Pont, Boston, MA) into a femoral vein. Glucose was clamped at basal by a variable glucose infusion labeled with D-[3-3H]glucose (NEN Research Products Du Pont, Boston, MA) into a femoral vein. Blood samples were taken every 10 min from 0 to 30 min. Samples for plasma FFAs were taken in EDTA and paraoxon to inhibit lipoprotein lipase, whereas all other samples were stored in tubes precoated with Li-NaF and heparin (Brinkmann Instruments, Westbury, NY).

Basal turnover design. At ~90 min, tracer glucose was infused (25 µCi/µmol, 0.25 µCi/min/µmol [3-3H]-glucose; NEN Research Products Du Pont) into a femoral vein. Blood samples were taken every 10 min from ~30 to 180 min. Samples for plasma FFAs were taken in EDTA and paraoxon to inhibit lipoprotein lipase, whereas all other samples were stored in tubes precoated with Li-NaF and heparin (Brinkmann Instruments).

Assays. Glucose was measured immediately after sampling on a YSI 2700 autoanalyzer (Yellow Springs Instruments, Yellow Springs, OH). Samples for [3H]-tracer assay were deproteinized using barium hydroxide and zinc sulfate. The supernatants were then evaporated in a vacuum, reconstituted in water, and counted in Ready Safe scintillation fluid (Beckman Liquid Scintillation Fluid; Beckman Instruments, Fullerton, CA). Tracer infusates were processed identically to plasma samples. Insulin was measured by an enzyme-linked immunosorbent assay originally developed for human serum or plasma by Novo Nordisk and adapted for dog plasma. The method is based on two murine monoclonal antibodies that bind to different epitopes on insulin but not to proinsulin. Materials for the insulin assay, including the dog standard, were kindly provided by Novo Nordisk. FFAs were measured using a kit from Wako (NEFA C; Wako Pure Chemical Industries, Richmond, VA), which uses a colorometric assay based on the acylation of coenzyme A. β-Hydroxybutyrate was measured with a kit based on the formation of NADH (Sigma-Aldrich, St. Louis, MO).

Glycerol enrichment was determined by gas chromatography-mass spectrometry (GC-MS) analysis using standard methods. Briefly, a [2-13C]-glycerol internal standard was added to samples, which were then deproteinized with ice-cold acetone. After drying, a 2:1 mixture of propionic anhydride and pyridine was added to derivatize the samples for GC-MS analysis. After drying, samples were reconstituted with ethyl acetate and analyzed by GC-MS (Hewlett Packard 6890/5973) using the electron impact mode and using a SP17 column (30 m × 0.25 mm inner diameter, 0.25-µm phase thickness).

Plasma palmitate specific activity was determined using a previously published method with slight modification (14). Briefly, 100 µl marginic acid internal standard (5 mmol/l in isopropanol) was added to 500 µl plasma samples. Fatty acids were isolated from water-soluble components of plasma with a Dolichloric acid precipitation, scaled up for use, and the increased sample size (14). One milliliter of the organic phase was subjected to counting in a Liquid Scintillation Cocktail (Beckman Instruments). A total of 200 µl of the organic phase was subjected to derivatization at 65°C with 400 µmol/l-p-bromophenacylbromide and 40 µmol/l 18-crown-6 in acetonitrile. Cold palmitate was measured by separating fatty acids on a 4.6 × 250 mm C6 Analytical HPLC Column (Spherisorb; Waters, Milford, MA) in the reverse phase with an initial 40:60 acetonitrile:water ratio and a 1% per minute gradient to 100% acetonitrile at 0.7 ml/min. Chromatographs were detected at 254 nm.

Deuterated water was used as a tracer for gluconeogenesis according to the method of Landau et al. (15). Incorporation of deuterium at the sixth carbon of glucose was monitored, with the caveat that glycerol gluconeogenesis, incorporated at the triose phosphate level, does not label the C6 position and is incorrectly quantified as glycogenolysis by this method. A recent study in preterm human infants indicates that gluconeogenesis by the deuterium oxide-C6 method is underestimated by the fraction accounted for by glycerol gluconeogenesis, as estimated by [2-13C]-glycerol infusion (16). This finding suggests that in premature infants with rapid water turnover, equilibration in the tricarboxylic acid cycle is nearly complete, and a very small fraction of unequilibrated oxaloacetate is converted directly to phosphoenolpyruvate. Thus, the deuterium oxide-C6 method provides an accurate method of quantifying gluconeogenesis with the caveat that glycerol gluconeogenesis is not included.

Deuterated water concentration in plasma was measured by isotope ratio mass spectrometry ( Finnigan Delta-E; Finnigan MAT, San Jose, CA) after reduction to hydrogen gas according to accepted methods (17,18). Deuterolysis was measured in hexamethylenetramine by GCMS using a Hewlett Packard 5973 Quadrupole instrument with an HP 5 column (25 m × 0.25 µm × 1.0 µm) in the electron impact mode with selected monitoring at 140 and 141 m/z as previously published (19).

Calculations. Steady-state values are taken as the average of four measurements from ~30 to 0 min for the zero insulin dose and from 150 to 180 min for all other insulin doses. EGP and glucose utilization (rate of disposal [Rg]) were calculated using Steele’s model with a labeled glucose infusion as detailed previously (13). Steady-state glycerol rate of appearance was calculated as previously described (20) with the following tracer dilution equation:

\[ R_{ap} = \frac{F}{IE \times 0.01} \]

where \( R_{ap} \) is the rate of appearance of glycerol, \( F \) is the infusion rate in milligrams per minute, and \( IE \) is the plasma isotopic enrichment expressed as a percentage.

The steady-state rate of appearance was calculated as previously described (20) with the following tracer dilution equation:

\[ R_{pal} = \frac{F}{SA} \]

where \( R_{pal} \) is the rate of appearance of palmitate, \( F \) is the tracer infusion rate, \( DPM \) is the disintegrations per minute, and \( SA \) is the specific activity in

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plasma. Total FFA rate of appearance \([R_{a}(FFA)]\) may be estimated by assuming that all FFAs behave as palmitate and correcting for the fraction of total plasma FFA represented by palmitate.

Intracellular FFA re-esterification rate was calculated as previously described (20) with the following:

\[
3 \times R_{a}(gly) - R_{a}(FFA)
\]

Gluconeogenesis, as a fraction of total glucose production, was calculated with a correction for exogenous glucose infusion, as described in the Appendix. Calculations using differential equations were performed with MLAB (Civilized Software, Bethesda, MD) implemented on an IBM-compatible computer.

**Statistical analysis.** Values are reported as means ± SE. Analysis of variance (ANOVA) was performed to test for an effect of troglitazone treatment on all measured parameters. Paired \(t\) tests were performed at each level of insulin when significance was achieved by ANOVA. All statistical calculations were performed using Minitab statistical software (State College, PA) implemented on an IBM-compatible computer.

**RESULTS**

**Glucose, glucose specific activity, and insulin.** Glucose was clamped at basal (95.1 ± 1.1 mg/dl; average coefficient of variation of 6.4%). Neither basal glucose nor basal insulin differed before versus after troglitazone treatment (\(P > 0.55\) and \(P > 0.52\), respectively). Glucose specific activity, when pooled across all experiments, did not vary between the initial and final steady-state periods (data not shown; \(P > 0.62\)). As predicted from previous studies (11), systemic insulin was matched for the HPO and IPE doses of insulin. Insulin levels for these two protocols were 151 ± 8 and 174 ± 18 pmol/l (HPO vs. IPE; \(P > 0.38\)) during the control period and 147 ± 10 and 158 ± 21 pmol/l (\(P > 0.92\)) after troglitazone treatment.

**FFA.** Plasma FFA concentrations are shown in Table 1. Despite lack of change in basal parameters, treatment with troglitazone sensitized the adipocyte to insulin. Plasma FFAs at zero insulin were unchanged after troglitazone treatment (0.64 ± 0.07 vs. 0.74 ± 0.11 mmol/l, \(P = 0.32\), paired \(t\) test). Suppression of FFAs in comparison with zero insulin (Fig. 1) was found to be enhanced by troglitazone treatment (55 ± 9 vs. 80 ± 6% for LPO, 84 ± 5 vs. 88 ± 5% for HPO, \(P < 0.01\), ANOVA). There was a substantial enhancement of insulin inhibition of FFAs at the intermediate insulin dose (0.28 ± 0.05 vs. 0.12 ± 0.02 mmol/l, \(P < 0.05\), \(t\) test), resulting in a leftward shift in the insulin/FFA dose-response curve. There was no significant effect of troglitazone at HPO (0.10 ± 0.03 vs. 0.07 ± 0.03 mmol/l, \(P = 0.54\), \(t\) test), likely because of complete suppression of adipocyte lipolysis. FFA oxidation was likely not changed by troglitazone treatment because plasma ketones were not different after troglitazone treatment (data not shown).

**Lipid turnover.** Because of the measurable difference in adipocyte insulin sensitivity with LPO administration, palmitate and glycerol turnover were characterized at this insulin dose before and after troglitazone treatment. FFA release into plasma and intracellular FFA re-esterification rates are shown in Fig. 2. Before troglitazone treatment, palmitate rate of appearance \((R_{a})\) was 1.2 ± 0.2 µmol · kg⁻¹ · min⁻¹; with...
troglitazone, mean palmitate $R_a$ was reduced 33% to 0.8 ± 0.2 µmol · kg$^{-1}$ · min$^{-1}$, although this change did not achieve significance. Palmitate averaged 23.8 ± 0.7% of total plasma FFA turnovers, and this percentage did not differ after troglitazone treatment. Therefore, total FFA turnover was estimated to be 6.7 ± 1.1 µmol · kg$^{-1}$ · min$^{-1}$ before troglitazone treatment and was reduced to 4.2 ± 0.9 µmol · kg$^{-1}$ · min$^{-1}$ after troglitazone treatment, supporting an effect of troglitazone to enhance adipocyte insulin sensitivity and reduce lipolysis. We did not detect a significant change in glycerol turnover (3.0 ± 0.3 vs. 2.8 ± 0.5 µmol · kg$^{-1}$ · min$^{-1}$ [NS]). Thus, there was an apparent increase in intracellular fatty acid recycling, calculated as three times the glycerol turnover minus the fatty acid turnover in plasma (4.3 ± 0.4 µmol · kg$^{-1}$ · min$^{-1}$ before troglitazone treatment to 5.0 ± 1.1 µmol · kg$^{-1}$ · min$^{-1}$ after troglitazone treatment [NS]), suggesting that adipocyte storage of FFAs was increased by troglitazone treatment in normal animals.

**Glucose turnover.** Rates of glucose infusion necessary to clamp plasma glucose are shown in Table 1. Glucose uptake as a function of insulin dose is shown in Table 1. Enhanced glucose uptake was not detected at any insulin dose after troglitazone treatment, despite the enhanced adipocyte insulin sensitivity. This result is not surprising because glucose uptake is stimulated little at the low insulin doses used in this study (8). In contrast, there was an effect of troglitazone to increase EGP at matched insulin levels. Rather than being reduced in parallel with FFAs, EGP exhibited a paradoxical increase after troglitazone treatment. This increase in liver production was observed at every insulin dose ($P<0.01$, ANOVA) and appeared to be independent of insulin because the zero insulin dose demonstrated a rise in EGP (2.1 ± 0.4 vs. 2.5 ± 0.4 mg · kg$^{-1}$ · min$^{-1}$, $P<0.05$, t test). Insulin suppression of EGP was affected as well, and this was most prominent at the highest insulin dose, where the EGP was suppressed by 90% in control studies but only 64% after troglitazone treatment.

**Gluconeogenesis.** Plasma water enrichment due to the deuterated water bolus averaged 2.50 ± 0.07%. In the calculation of gluconeogenesis, a correction was required to account for the plasma glucose derived from the exogenous infusion, which was not deuterated in the C6 position of glucose (see Appendix). At steady state, the ratio of plasma glucose derived from the glucose infusion to plasma glucose derived endogenously averaged 2.6 ± 0.2 before troglitazone treatment and 2.2 ± 0.2 after troglitazone treatment (NS, t test). Gluconeogenesis was then calculated during the steady-state period of the LPO insulin dose (Table 1 and Fig. 3). It was expected that with reduced plasma FFAs, gluconeogenesis would be decreased as well (21,22). However, like EGP, gluconeogenesis increased after troglitazone treatment (0.6 ± 0.1 vs. 0.9 ± 0.1 mg · kg$^{-1}$ · min$^{-1}$ before and after troglitazone treatment; $P<0.05$, t test). Additionally, glycogenolysis, calculated as EGP minus gluconeogenesis, was significantly increased after troglitazone treatment (0.4 ± 0.1 vs. 0.6 ± 0.1 mg · kg$^{-1}$ · min$^{-1}$ before and after troglitazone treatment, respectively; $P<0.05$). Therefore, the rise in EGP was apparently the result of both elevated gluconeogenesis and elevated glycogenolysis, and the fraction of glucose production from gluconeogenesis and glycogenolysis was not altered by troglitazone treatment (NS).

**Portal versus peripheral insulin infusion.** We have previously reported that as long as peripheral insulin concentrations are matched, there is little difference in suppression of EGP, regardless of whether insulin is administered portal or peripherally. This independence of portal insulin was maintained in the present study, where both FFAs (0.10 ± 0.01 vs. 0.16 ± 0.06 mmol/l; $P>0.28$) and EGP (0.2 ± 0.3 vs. 0.9 ± 0.2 mg · kg$^{-1}$ · min$^{-1}$; $P>0.86$) were similar when comparing the HPO and IPE insulin doses during the control period. After troglitazone treatment, the dominance of peripheral insulin remains, where FFAs (0.07 ± 0.02 vs. 0.07 ± 0.01 mmol/l; $P>0.69$) and EGP (0.9 ± 0.2 vs. 1.2 ± 0.2 mg · kg$^{-1}$ · min$^{-1}$; $P>0.06$) were similar.
DISCUSSION

The agent troglitazone has been shown to have potent effects on peripheral insulin sensitivity in insulin-resistant subjects, including not only subjects with diabetes but also postpartum gestational diabetic subjects in whom fasting blood glucose has renormalized. In addition, it has been suggested that thiazolidinediones may retard or even prevent the onset of diabetes (23). Thus, it is important to understand troglitazone’s actions in normal individuals who may someday be treated with troglitazone or other PPAR-γ agonists as a preventive measure for diabetes and other concomitants of the insulin resistance syndrome.

In contrast to previous reports, this study demonstrated that thiazolidinediones modify fat and carbohydrate metabolism in normal animals. Clearly, the adipocyte was sensitized to insulin action after treatment with troglitazone. The Randle hypothesis suggests that FFA oxidation and glucose oxidation are inversely related. Therefore, it was expected in this study that a reduction in FFA availability secondary to enhanced insulin action would enhance glucose uptake. Additionally, the single gateway hypothesis, posited in our laboratory, emphasizes the tight relationship between plasma FFA and glucose production. The a priori expectation of these studies was that, if adipocyte insulin sensitivity were enhanced by troglitazone, then the consequential reduction in plasma FFAs would be anticipated to lower EGP.

It was found that plasma FFAs were indeed more suppressed at matched insulin after troglitazone treatment. Without insulin replacement, plasma FFAs were not affected, suggesting that lipolysis independent of insulin was not affected. At the lesser insulin infusion, which elevated insulin to ~50 pmol/l, FFAs were suppressed only 44% without troglitazone treatment but were almost completely suppressed with troglitazone treatment. At the higher portal dose, raising insulin to ~150 pmol/l, again there was no difference in plasma FFAs, presumably because of almost complete suppression of lipolysis (8). Glycerol turnover was not affected by troglitazone treatment, suggesting that the reduced rate of lipolysis was not responsible for the suppressed plasma FFAs at the intermediate dose of insulin. In contrast, palmitate turnover had a tendency to be reduced after troglitazone treatment. The lack of suppression of lipolysis and the trend for increased palmitate turnover supports the concept that in normal animals, troglitazone increased intracellular re-esterification, sparing FFAs from the circulation. This latter concept is in concordance with previously published experiments performed in vitro, where fatty acid storage was also found to be increased (24). Although increased intracellular re-esterification in this study did not reach statistical significance, the data are consistent with the concept that thiazolidinediones “keep the fatty acids in the fat cell” (24).

Glucose uptake was not increased by troglitazone treatment of normal animals. This fact is in contrast with a plethora of data on insulin-resistant animals and humans in whom glucose disposal is improved by thiazolidinediones. The Randle hypothesis would predict that suppressed plasma FFAs could lead to increased rates of glucose uptake. However, the low insulin concentrations at which FFAs were affected were too low to stimulate glucose uptake. At the higher insulin dose, FFAs were actually similar because lipolysis was fully suppressed. Therefore, it is reasonable that a difference in glucose uptake would not be detected. Under conditions of insulin resistance and, in particular, visceral obesity, in which there is an expansion of insulin-resistant fat cells, FFAs may be a more important determinant of glucose uptake under hyperinsulinemic conditions.

This experiment was designed to maximize the indirect effect of insulin on glucose production. A previous study from our laboratory indicates that under conditions of hypoglycagonecemia, this indirect effect is enhanced (11). Thus, in these studies, glucagon secretion was suppressed with somatostatin and not replaced. In contradistinction from the expected result based on the single gateway hypothesis, EGP did not fall, despite the decline in plasma FFAs. Surprisingly, EGP was paradoxically higher after troglitazone treatment. In addition, the rate of gluconeogenesis did not decline. This result was unexpected given that other studies, although in vitro, have shown that FFAs control the gluconeogenic rate (22). Furthermore, the fraction of glucose production from gluconeogenesis and glycogenolysis remained unaltered after troglitazone treatment, suggesting that the effect of the drug was not specific to either pathway. To understand this paradox, it is necessary to consider the consequences of an increase in insulin sensitivity of the liver in normal animals. In fact, such an increase would result in hypoglycemia because the fasting rate of glucose production would fall in the face of equal or increased peripheral insulin sensitivity. Therefore, one interpretation of our data is that there was a re-regulation of the sensitivity of EGP to plasma FFAs that may have occurred in these normal dogs to prevent hypoglycemia. This speculation is further supported by the lack of complete suppression of EGP after troglitazone treatment at the highest dose of portal insulin infusion. This re-regulation is depicted in Fig. 4, which shows that the relationship between EGP and FFAs is altered by troglitazone treatment.

Portal infusions of insulin at the high dose were compared with peripheral insulin infusions designed to match the systemic insulin levels. Because of the high extraction rate of insulin by the liver, this protocol resulted in similar systemic insulin concentrations but differing portal insulin concentrations. The time course and steady-state rates of EGP were similar in the control situation, confirming previous studies that peripheral insulin is much more important than portal insulin in controlling EGP. Thus, the importance of systemic insulin in controlling EGP remains dominant after troglitazone treatment.

To our knowledge, this is the first demonstration of a chronic effect of troglitazone in normal animals. An acute study in normal rats demonstrated that troglitazone infusion had a rapid effect on glucose metabolism, suppressing glucose production and increasing glucose uptake within 60 min (25). The first measurable changes in glucose infusion rate were within 30 min, suggesting that the effect was not due to a regulation of protein abundance. This result implies that troglitazone may have metabolic effects outside of PPAR-γ, or activation of PPAR-γ may induce rapid changes not related to gene expression. It has been suggested that troglitazone may have extra-PPAR effects due to the tocopherol moiety covalently bound to the thiazole ring. Thus, troglitazone may be nonspecific toward PPAR-γ.
It should be noted that normal time controls were not included in this study. It has been previously demonstrated that, in our hands, glucose tolerance is reduced with duration of time in the vivarium by 20 ± 6% over the course of 3 weeks (26). However, this was associated with a reduction in acute insulin response to glucose of 23%, suggesting a lack of change in insulin sensitivity. If insulin secretion was the only parameter modified by time alone, then it may be said with confidence that changes found in this study are due to drug treatment per se because the clamps are performed under hypersomatostatinemia. Furthermore, it is unlikely that an increase in adipocyte insulin sensitivity would occur because of the relatively inactive lifestyle of the animals in the vivarium. Therefore, it is unlikely that the findings of this study are due to time spent in the vivarium.

In conclusion, troglitazone was effective in improving adipocyte sensitivity to insulin in normal dogs. This finding was evidenced by increased suppression of FFAs within the physiological range. EGP did not fall with the suppression of FFAs—a finding in conflict with the single gateway hypothesis. It is hypothesized that the sensitivity of EGP to FFAs was modified to prevent hypoglycemia that would result if EGP declined with FFAs.

APPENDIX

Because an undeuterated exogenous glucose infusion (GINF) was used in this study to clamp plasma glucose at basal levels, the enrichment of C6 glucose in plasma from gluconeogenesis was diluted by the undeuterated exogenous glucose. Furthermore, GINF was variable throughout the experiments, including to some extent the steady-state periods, to exquisitely clamp the plasma glucose. Using the values of steady-state GINF to correct for the dilution of endogenous glucose resulted in an overestimation of the gluconeogenic rate because the glucose pool had not completely turned over by the end of the steady-state period. A detail of the corrections for GINF dilution follows.

In plasma, the proportion of glucose from GINF was calculated so the proportion of EGP derived from gluconeogenesis could be determined by subtraction. A one-compartment model of glucose kinetics using Steele’s volume was used to calculate these proportions (Fig. 5), with $p = 0.65$ and $V = 25\%$ of body weight.

The mass of glucose from each source are as follow:

$$\frac{dG_{\text{exo}}(t)}{dt} = GINF(t) - R_d(t) \frac{G_{\text{exo}}}{G_{\text{tot}}}$$

$$\frac{dG_{\text{endo}}(t)}{dt} = EGP(t) - R_d(t) \frac{G_{\text{endo}}}{G_{\text{tot}}}$$

where $G_{\text{exo}}$ is the glucose in plasma from exogenous sources, $G_{\text{endo}}$ is the glucose in plasma from endogenous sources, $G_{\text{tot}}$ is the total glucose in plasma ($G_{\text{endo}} + G_{\text{exo}}$), $GINF(t)$ is the exogenous glucose infusion rate, and $R_d(t)$ and $EGP(t)$ are the rates of disappearance and appearance of glucose, respectively, based on [3-3H]glucose dilution.

By setting an initial point for the differential equations, the time course of $G_{\text{endo}}$ and $G_{\text{exo}}$ could be reconstructed based on known $GINF(t), R_d(t)$, and $EGP(t)$. The initial point was based on the assumption that before any GINF infusion, 100% of the glucose in plasma originated from EGP.

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